Peptide Complex

First Hit

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REPRESENTATIVE-FIGURES: NONE

ABSTRACT:

The invention provides multimeric receptor-binding complexes, including chemokine tetramers, useful for recognizing and binding receptors bound to the surface of a wide variety of cells. The binding complexes are useful for identifying and isolating cells according to their specific receptors, screening for cells having a specific receptor or constellation of receptors, and introducing exogenous molecules (e.g., nucleic acids and toxins) into cells. Methods of producing the complexes and other uses are also described.

[0001] The present application claims the benefit of U.S. S. No. 60/360,724, which was filed on Feb. 27, 2002. The contents of that provisional application are hereby incorporated by reference in their entirety.

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Abstract Paragraph:
The invention provides multimeric receptor-binding complexes, including chemokine tetramers, useful for recognizing and binding receptors bound to the surface of a wide variety of cells. The binding complexes are useful for identifying and isolating cells according to their specific receptors, screening for cells having a specific receptor or constellation of receptors, and introducing exogenous molecules (e.g., nucleic acids and toxins) into cells. Methods of producing the complexes and other uses are also described.

Summary of Invention Paragraph:
[0007] Methods of making the complexes and other compositions of the invention are described further below, as are methods of using them to deliver moieties (e.g., detectable labels, nucleic acids, other therapeutic agents, or toxins) to, for example, a cell that expresses the cognate receptor. For example, a complex including nerve growth factor (NGF) as the receptor-binding molecule can be used to detect an NGF receptor or deliver a moiety to an NGF receptor-bearing cell; a complex including interleukin 2 (IL-2) as the receptor-binding molecule, can be used to detect an IL-2 receptor or deliver a moiety to an IL-2 receptor-bearing cell; a complex including CCL19 as the receptor-binding molecule can be used to detect a CCL19 receptor (CCR7) or deliver a moiety to a CCR7-bearing cell; etc. In any case, the detection or delivery can be carried out for diagnostic or therapeutic reasons (particular assays and treatments are described further below). The complexes can also be used to identify therapeutic agents. For example, a potential therapeutic agent can be incorporated into the complex (in addition to, or in place of, one or more of the "receptor-binding molecules" described below), and brought into contact with a biological cell (in culture or in vivo). One can then assess the cellular response (or, if the assay is carried out in vivo, one can assess signs or symptoms of disease in the animal) to determine whether the agent exerts a desired effect. The precise parameter assessed can vary, depending on the agent tested and its expected application.

Summary of Invention Paragraph:
[0009] While immunocytochemistry can presently be used to identify cells bearing many types of receptors, antibodies that specifically bind all of the known receptors or receptor-binding ligands are not available. The complexes described herein allow one to detect receptors in such cases (i.e., where antibodies are not available or are less than optimal). Where the specificity of the interaction between the complexes of the invention and their cognate receptor is high, the complexes can be used to target cells that express a particular cytokine receptor. This is advantageous because it may increase the specificity with which nucleic acid molecules, other therapeutic agents, or other types of molecules (such as toxins) can be delivered to a patient. In addition, when compared to the binding of monomeric ligands, the complexes of the invention may be more stable; they can, for example, have an increase in t1/2 that is 5-, 10-, 15-, 20-, or even 50-fold higher than for uncomplexed signaling molecules.

Detail Description Paragraph:
[0044] The receptor-binding molecule can also be a hormone, neurotransmitter, or co-stimulatory molecule and, as with the other types of receptor-binding molecules described above, can be a biologically active fragment or other mutant (e.g., substitution mutant) of such molecules. The hormone can be a hormone produced by the adrenal gland, parathyroid gland, pituitary gland, or thyroid gland; it can also be produced by the hypothalamus, the ovary, the testicle, the pancreas, the pineal body, or the thymus. For example, the hormone can be a thyroid-stimulating hormone, a follicle-stimulating hormone, a leuteinizing hormone, prolactin, growth hormone, adrenocorticotropic hormone, antidiuretic hormone, oxytocin, thyrotropin-releasing hormone, gonadotropin-releasing hormone, growth hormone-

releasing hormone, corticotropin-releasing hormone, somatostatin, dopamine, melatonin, thyroxine, calcitonin, parathyroid hormone, a glucocorticoid, a mineralocorticoid, an androgen, adrenaline, an estrogen, progesterone, human chorionic gonadotropin, insulin, glucagons, somatostatin, erythropoietin, calcitriol, atrial-natriuretic peptide, gastrin, secretin, cholecystokinin, somatostatin, neuropeptide Y, ghrelin, PYY.sub.3-36, insulin-like growth factor-1, angiotensinogen, thrombopoietin, or leptin.

Detail Description Paragraph:
[0054] As noted above, the linkers and multivalent binding partners can be varied, and their selection can depend on how strongly bound or stable one wishes the complex to be. For example, Strep-Tag.RTM. II binds Strep-Tactin.RTM. with an affinity that is about 100 times greater than its affinity for streptavidin (IBA, St. Louis, Mo.). Thus, if one desired a weaker interaction between the receptor-binding molecule and the multivalent binding partner, the receptor molecule could be fused to Strep-Tag.RTM. II and then complexed with streptavidin. Weaker interactions may be preferable when one wishes the complex to dissociate; dissociation can facilitate the activity of a cargo molecule, such as a toxin or nucleic acid, that is delivered to a cell by way of attachment to the multivalent protein, receptor-binding molecule, or other part of the multimeric complex. To the contrary, tighter associations may be preferable when the stability of the complex is more important. For example, tighter association may be preferable when a multivalent binding partner (or other member of the complex) is fused to a detectable label, such as a fluorophore, for the purpose of labeling a cell.

Detail Description Paragraph:
[0073] Consistent with our discussion above, the crosslinking agent can be selected based on the strength of the association desired. For example, a cross-linker that functions by creating disulfide bonds (e.g., DTSSP) between members of the complex can be used to generate a complex that will disassemble upon uptake into a cell; upon internalization, the bonds are reduced, thereby releasing a cargo, such as a toxin or nucleic acid, that is attached to the complex. The strategies for complex disassembly that are used for synthesis of immunotoxins can be used in the context of the present invention (see, for example, Thorpe and Ross, Immunol. Rev. 62:119-158, 1982; Thorpe et al., Cancer Res. 47:5924-31, 1987; and Myers et al., Immunol. Methods 136:221-37, 1991).

Detail Description Paragraph:
[0097] The expanded complex can be brought into contact with a target cell in a variety of ways. For example, the two can be incubated together in culture or in solution. Alternatively, the complex can be administered to a patient (routes of administration are described below) or applied to a patient's tissue (in vivo or following explantation (e.g., prior to re-implantation or transplantation)). While the invention is not limited to compositions that act by any particular mechanism, or to methods in which any particular event occurs, it is thought that complexes containing a therapeutic moiety are taken into the cell by endocytosis. Depending on the cargo, dissociation of the cargo and the multimeric complex is not always necessary to elicit a desired effect on a cell. For example, some cytotoxins can induce cell death even when the toxin is still attached to the multimeric complex. In another strategy, the cargo can be attached to the receptor-binding molecule by a disulfide linkage. Upon internalization of the complex, the disulfide bond is reduced, and the cargo is released from the complex. This strategy is commonly used for the development of immunotoxins (see, for example, Thorpe and Ross, Immunol. Rev. 62:119-158, 1982; Thorpe et al., Cancer Res. 47:5924-31, 1987; and Myers et al., Immunol. Methods 136:221-37, 1991). Another strategy to facilitate dissociation of the cargo from the complex is to use a linker/multimeric binding complex combination that has a higher dissociation constant (K.sub.D; i.e., lower binding affinity) than other combinations. For example, as discussed supra, the Strep-Tag II binds Strep-Tactin with an affinity that is 100 times greater than its affinity for streptavidin (IBA, St. Louis, Mo.). The cargo can be fused to Strep-Tag II and then complexed with streptavidin. The faster off-rate will facilitate release of the cargo from the complex. A similar strategy can be employed in designing methods to deliver a cargo to a cell by attachment of the cargo to the receptor molecule instead of to the

multivalent binding partner. In some cases it may be preferable to couple the cargo to the multivalent binding partner (rather than to the receptor molecule) to avoid interference of the cargo with receptor-binding molecule/receptor interactions.

Detail Description Paragraph:
[0100] As noted, the complexes can also be used to deliver cytotoxins, which can include cytotoxic antibodies, polysaccharides or chemical compounds (e.g., small molecules), to cells expressing defined receptors. Genes encoding proteins that trigger apoptosis are also cytotoxic agents. Specific cytotoxins, any of which can be incorporated in the complexes of the invention, include ricin, abrin, diptheria toxin, maytansinoids, cisplatin, and the like. Where there are two subunits, only the cytotoxic subunit may be used (e.g., the .alpha.-unit of ricin). The toxin (or other agent slated for delivery) can be conjugated to the binding complex in a variety of ways. For example, it can be joined by means of a cross-linker or by way of a disulfide bond. Toxin conjugates are disclosed in, for example, U.S. Pat. Nos. 5,208,020; 4,863,726; 4,916,213; and 5,165,923.

CLAIMS:

14. The multimeric complex of claim 11, wherein the hormone is a thyroid-stimulating hormone, a follicle-stimulating hormone, a leuteinizing hormone, prolactin, growth hormone, adrenocorticotropic hormone, antidiuretic hormone, oxytocin, thyrotropin-releasing hormone, gonadotropin-releasing hormone, growth hormone-releasing hormone, corticotropin-releasing hormone, somatostatin, dopamine, melatonin, thyroxine, calcitonin, parathyroid hormone, a glucocorticoid, a mineralocorticoid, an androgen, adrenaline, an estrogen, progesterone, human chorionic gonadotropin, insulin, glucagons, somatostatin, erythropoietin, calcitriol, atrial-natriuretic peptide, gastrin, secretin, cholecystokinin, somatostatin, neuropeptide Y, ghrelin, PYY.sub.3-36, insulin-like growth factor-1, angiotensinogen, thrombopoictin, or leptin.